Body size, age structure and survival rates in two populations of the Beyşehir frog *Pelophylax caralitanus*

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Abstract

In many amphibians, skeletochronology is a reliable tool for assessing individual mean longevity, growth rates and age at sexual maturity. We used this approach to determine the age structure of 162 individuals from two *Pelophylax caralitanus* populations. All individuals exhibited Lines of Arrested Growth (LAGs) in the bone cross-sections and the average age varied between 4.5 and 5.4 years in both Işıklı and Burdur populations. Although intraspecific age structure and sex-specific age structure did not differ significantly between populations, we found that the Işıklı population had a lower body size in the same age class, had lower growths rates and lower values of survival rates and adult life expectancy than the Burdur population.

Key Words

Introduction

Growth rate and body size are important intraspecific characteristics for adult amphibians. Skeletochronology is a reliable tool for assessing individual mean longevity, growth rates and age at sexual maturity (Castanet et al. 1993; Smirina 1994). Lines of Arrested Growth (LAGs) are annually developed in the long bones during periods of unfavourable growing conditions, such as winter and they allow accurate calculations for determining the age of anurans. This technique was successfully performed on the phalanges of many amphibian species, hence it represents a powerful technique (Olgun et al. 2005; Guarino and Erişmiş 2008; Üzüm and Olgun 2009; Üzüm et al. 2011; Altunışık and Özdemir 2013; Sinsch 2015). This technique is also used for other endangered species and allows individuals to be marked for field study and skeletal elements to be obtained for skeletochronology without sacrificing the individuals (Castanet and Smirina 1990; Guarino et al. 1999). In addition, this technique, which uses seasonally variable physiological activity, has proven to be an excellent tool in evaluating population age models in amphibian species (Esteban and Sanchiz 2000).

Pelophylax caralitanus, Beyşehir frog (Arıkan, 1988) was originally described as a nominant subspecies of *Pelophylax ridibundus* by Bodenheimer (1944). Arıkan (1988) described the Beyşehir population from Lake Beyşehir as a new subspecies (*R. r. caralitana*), based on morphometric characters. However, Beerli et al. (1994) claimed that *R. r. caralitana* is not a new subspecies and that *R. r. caralitana* and *Rana levantina* should be regarded as synonyms of *Rana [Pelophylax] bedriagae*. The subsequent karyological, morphological, genetic and bio-acoustical studies on the taxonomy of the Beyşehir population showed that *R. r. caralitana* is different from both *Pelophylax ridibundus* and *Pelophylax bedriagae* (Arıkan et al. 1994; Alpagut and Falakalı 1995; Budak et al. 2000; Kaya et al. 2002). As a result of taxonom-

ic assessments, *P. caralitanus* is an endemic species of Turkey and is distributed only within the Anatolian Lake District and Konya plain of Turkey (Jdeidi 2000; Plötner et al. 2001). Previous studies have shown that *P. caralitanus* lives in permanent wetlands with rich aquatic vegetation, including ponds, rain ponds, streams, rivers and irrigation canals (Arıkan 1988; Atatür et al. 1989–1990, Jdeidi 2000; Plötner et al. 2001; Kaya et al. 2002; Düşen et al. 2004; Başkale and Çapar 2016; Başkale et al. 2017). *Pelophylax caralitanus* has been listed as Near Threatened (NT) because of ongoing threats from habitat loss and over-exploitation (Öz et al. 2009).

The primary aim of our study was to determine the sex-specific variability of age and size at maturity and longevity of *P. caralitanus*. We also discuss the age distribution of a cognate species in different geographic ranges.

Materials and methods

Study sites

Our study was performed on Burdur Lake and Işıklı Lake. Both lakes, which have been fed by an underground water source, rain and other permanent water sources for many years, are natural habitats for amphibians.

The Burdur Lake population (37°38'N, 30°03'E; 859 m a.s.l.) is located adjacent to villages of Burdur province. Burdur Lake covers 250 km² and attains a maximum depth of 110 m. This area is surrounded by agricultural land and consists of a small wetland and a channel that is connected to Burdur Lake. The periphery and water body of this site is densely covered by aquatic vegetation. Agricultural activities, such as supplying water for irrigation and amateur fishing (for *Carassius gibelio*), are performed on this site.

Işıklı Lake population (38°12'N, 29°49'E; 820 m a.s.l.) is located in Çivril, Denizli province. Işıklı Lake covers 73 km² and has a maximum depth of 7 m. This area is surrounded by settlements and agricultural areas and consists of small streams and man-made channels. There is also an energy power plant on the southwest of the lake. The periphery and water body of this site are covered by aquatic vegetation. At irregular intervals, trees such as *Salix* sp. and broken branches of these trees have floated on the water surface. Agricultural activities, such as supplying water for irrigation, have caused a considerable decrease in the water level of the lake (up to 3 m) from July to September. Additionally, camping, picnic activities and amateur fishing (for *Esox lucius* and *Tinca tinca*) are undertaken on this site.

Field studies

Individuals of *P. caralitanus* were captured by two or three persons with a dip net or by hand after sunset using flashlights during the 2015–2018 breeding seasons. The Snout-Vent Lengths (SVL) were obtained with a dial caliper at a 0.02 mm accuracy. We determined the sex of the individuals, based on the secondary sexual organs: males have tubercules on the first finger of their front foot and a paired vocal sac on their head.

Laboratory studies

According to the skeletochronology study literature, to determine the age of the species, the longest digit of the hind foot was removed and fixed in 70% ethanol. The skeletochronology procedure followed the previous studies (Castanet and Smirina 1990; Castanet et al. 1993; Smirina 1994). The bones of each animal were cleaned from the tissues, washed in running water for 12–14 h, decalcified for 4–6 h in 5% nitric acid and then placed in distilled water overnight. The bones were dehydrated using graded ethanol and then cleared in xylene, before embedding in paraffin. Using a rotary microtome, we obtained 14–16 μm thick cross-sections from the central region of the diaphysis, stained them with Ehrlich's Haematoxylin and Eosin and analysed them under an Olympus CX31 light microscope that was equipped with a digital camera, Kameram 5. The age of the amphibian was determined by two of the authors who independently counted the number of Lines of Arrested Growth (LAGs) present in each of the bone sections.

Statistical analyses

The data were normally distributed (Kolmogorov-Smirnov D test, all P > 0.05), thus allowing comparisons using parametric tests. We used an independent samples t test to compare the sexes morphometrically. We assumed that the age at first reproduction (Age at Maturity: AM) is the lowest age recorded amongst the breeding individuals.

The sexual dimorphism index (SDI) was calculated as SDI = (mean length of the larger sex / mean length of the smaller sex) ±1. The plus-minus sign (±) gives +1 if males are larger than females, defining the result as negative and −1 if females are larger than males, defining the result as positive. This formula was generated by Lovich and Gibbons (1992).

Growth was estimated according to the Bertalanffy equation (Bertalanffy 1938) which was previously used in several studies on amphibians (Miaud et al. 2001; Gül et al. 2011; Üzüm et al. 2011; Erişmiş 2018). The modified growth formula is;

$$SVL_t = SVL_{max} - (SVL_{max} - SVL_{met})e^{-k(t-tmet)}$$

where SVL_t is the average body length at age t; SVL_{max} is the asymptotic maximum body length; SVL_{met} is the body length at metamorphosis that was used to calculated newly metamorphosed individuals at the end of the summer

(25–30 August 2018) (fixed to mean 32.7 ± 3.415 mm for the Burdur population and 31.9 ± 3.625 mm for the Işıklı population); k is the body growth rate coefficient (units are yr^{-1}) that defines the shape of curve; t_{met} is the age at metamorphosis (0.3).

The parameters SVL_{max} and k and their asymptotic confidence intervals (CI) were estimated by non-linear regression. Annual growth rate (AGR) is the rate of the difference between the mean SVL of the individuals in each age group i and the mean SVL in each age group i-1 (AGR= the mean SVL of the Age_i - the mean SVL of the Age_{i-1} / The mean SVL of the Age_{i-1}). This formula was obtained from Erişmiş (2018). All statistical analyses were conducted using SPSS ver. 20.0 (SPSS 2011).

The survival rates assume a constant survival rate across all age classes and sampling of individuals with respect to age, which were estimated from Robson and Chapman's (1961) formula: S= T/(R+T – 1) where S is the finite annual survival rate estimate, T is $N_1+2N_2+3N_3+....$ and R is ΣN_i, where N_i is the number of individuals in age group i.

Adult life expectancy (ESP) is the expected average age and differs from the longevity value, which is the highest recorded age amongst individuals. Adult life expectancy was derived from Seber's formula (Seber 1973): ESP= 0.5+1/(1-S) where S is the survival rate.

Results

A total of 162 individuals (Burdur Lake = 35♂, 35♀ and 13 subadults; Işıklı Lake = 34♂, 38♀ and 7 subadults) were examined in this study. All examined bones in adult frogs had well-defined LAGs and endosteal bones with visible layers (Figure 1). They appeared as thin and approximate concentric layers, more intensely stained than the rest of the cross-section.

The mean SVL was 84.12 ± 1.83 mm (mean ± SE; range = 55.2 - 115.4 mm, n = 73) in females and 76.67 ± 1.48 mm (range = 52.9 - 105.1, n = 69) in males. The mean SVL (t = 7.109, df = 139, P < 0.01) was significantly different between males and females which means that females were significantly larger than males. The body size comparisons according to ages were presented in Table 1. The sexual dimorphism index was calculated as 0.1, indicating a female bias. Furthermore, the SVL of individuals showed statistically significant differences between Işıklı and Burdur populations (t = 2.933, df = 70, P < 0.05 for females; t = 2.841, df = 67, P < 0.05 for males). These results showed that the highest SVL for both sexes were found in the Burdur population. These data were supported by the sex specific asymptotic size of individuals in both populations.

The mean ages of males and females were calculated as 5.2 ± 0.25 and 4.6 ± 0.22 years, respectively. Age, size and growth parameter variations of both populations are given in Table 2. The age distributions were not significantly different between sexes in both populations
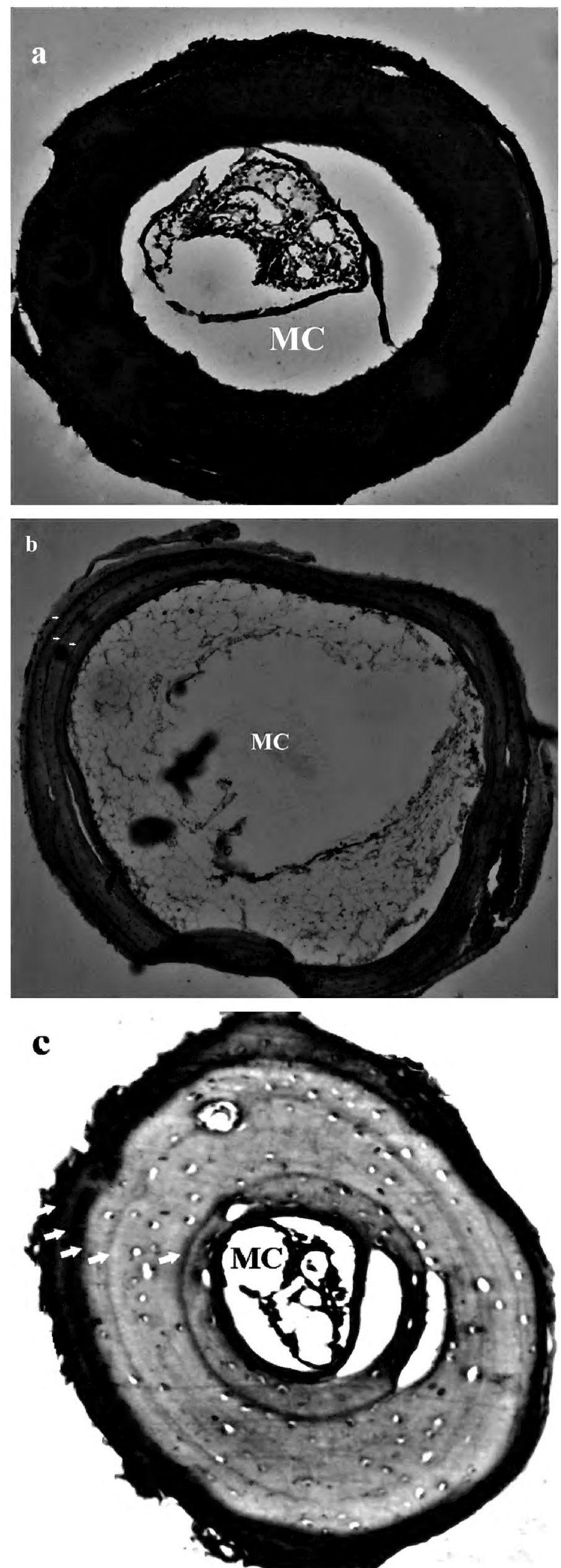

Figure 1. Phalangeal cross-sections (16 μm thick) of *P. caralitanus*. The white arrows show LAGs in the periosteal bone: (**a**) Newly metamorphosed individuals with 33.6 mm SVL Male: 3 LAGs with 56.3 mm SVL, (**b**) Female, 5 LAGs with 73.3 mm SVL. MC=Marrow cavity.

Table 1. Age and sex dependent body size differentiations of *P. caralitanus* in two populations.

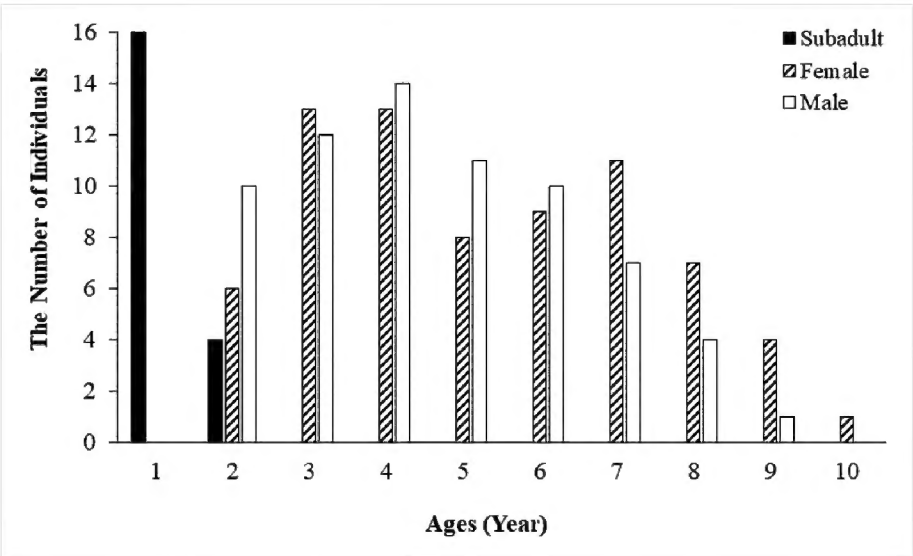|	Age	Newly Metamorphosed (MT)				Subadults (SA)				Female (F)				Male (M)			
		n	Min-Max	Mean SVL	S.E	n	Min-Max	Mean SVL	S.E	n	Min-Max	Mean SVL	S.E	n	Min-Max	Mean SVL	S.E
Burdur Population	0	22	24.9–39.0	32.7	0.72												
	1					11	35.8–49.8	43.9	1.36								
	2					2	55.0–65.0	60.0	5.00	4	53.0–63.0	57.0	2.12	6	53.0–68.0	58.5	2.35
	3									4	72.0–87.0	80.7	3.54	5	73.0–78.0	76.0	1.05
	4									6	76.0–89.0	83.1	1.99	7	75.0–89.0	81.5	2.02
	5									4	73.3–92.0	83.6	3.91	5	70.0–96.0	81.0	5.18
	6									5	84.0–102.0	93.4	3.44	5	76.0–99.0	88.4	4.20
	7									4	98.0–105.0	101.7	1.65	4	86.0–97.0	92.5	2.40
	8									5	102.0–110.0	106.0	1.52	2	96.0–102.0	99.0	3.00
	9									2	112.0–115.4	113.7	1.70	1	105.0	105.0	
	10									1	113.0	113.0					
Işıklı Population	0	7	27.0–37.0	31.8	1.37												
	1					5	37.0–48.0	42.0	1.92								
	2					2	51.8–52.2	51.9	0.21	2	65.0–68.0	66.5	1.50	4	57.9–65.0	62.0	1.59
	3									9	58.6–77.0	67.2	2.56	7	58.0–69.0	64.4	1.64
	4									7	62.0–82.0	73.2	2.78	7	65.0–79.0	70.5	1.96
	5									4	77.0–88.0	82.8	2.29	6	69.3–84.0	75.7	1.96
	6									4	73.3–87.0	80.1	2.83	5	71.1–88.0	77.9	2.81
	7									7	80.8–95.0	89.0	1.79	3	82.0–86.0	84.2	1.18
	8									2	92.9–104.2	98.5	5.69	2	86.7–92.0	89.3	2.67
	9									2	96.4–110.7	103.5	7.16				

Figure 2. Age distributions of *P. caralitanus* from the two populations.v.

(Burdur; t = 1.520; df = 68; p > 0.05, Işıklı Lake; t = 1.120; df = 69; p > 0.05). Furthermore, the sex-specific age structure did not differ significantly between populations (t = 0.800; df = 70; p > 0.05 for females and t = 0.886; df = 67; p > 0.05 for males). The age distribution is shown in Figure 2. Our results showed that maturity ages were two or three years in both sexes. Longevity of males was eight years in the Işıklı population and nine years in the Burdur population, whereas in females, it was nine years in Işıklı and 10 years in Burdur. The mean ESP of females and males was estimated as 6.61 and 6.15 years, respectively. Similarly, the mean survival rates of females and males were calculated as 0.84 and 0.83, respectively. Growth patterns of the two populations were compared according to the von Bertalanffy growth model (Figure 3). We found that the growth coefficients were higher in females than in males, while the peak growth
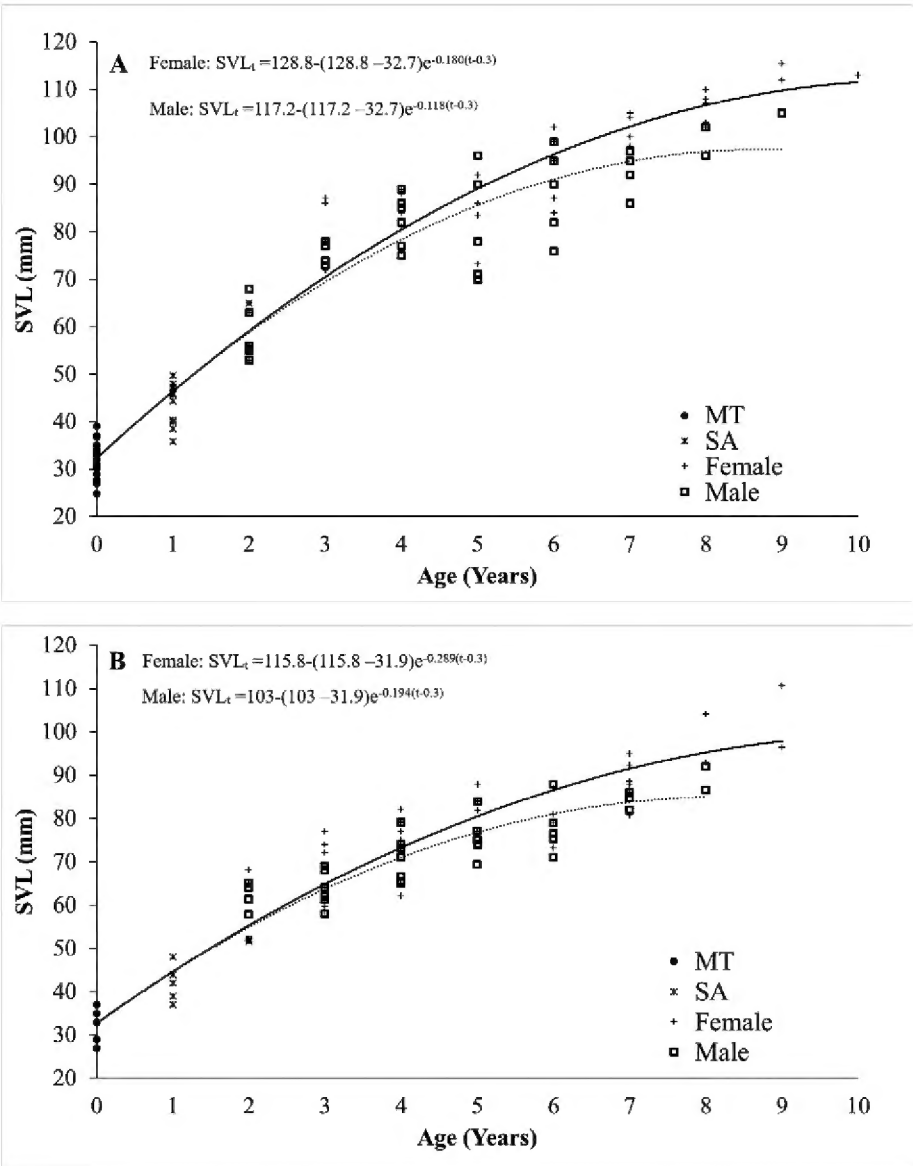

Figure 3. Relationship between SVL and age in Burdur population (**A**) and Işıklı population (**B**) of *P. caralitanus*, as expressed by Von Bertalanffy growth curves. MT: Newly Metamorphosed, SA: Subadults.

rate was found to be in the age range of 1–2 years. This slowly decreased after that to the age range of 3–4 years in both sexes (Figure 4).

Table 2. Comparison of age, size and growth parameters of *P. caralitanus* in two populations and cognate species: a comparison with existing literature. (AM: Age at Maturity, k: Growth coefficient, ESP: Adult life expectancy, SR: Survival rate).

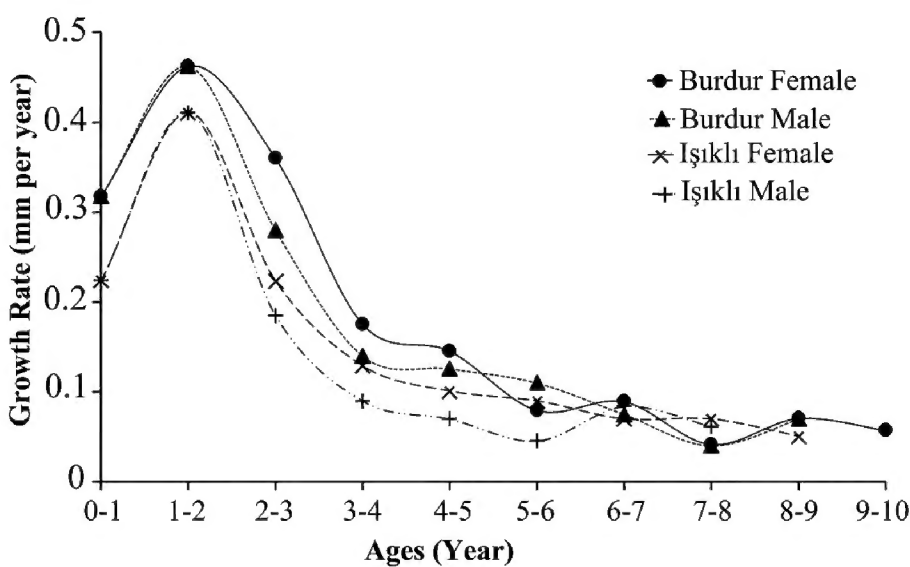| Species	Locality	Sex	N	Mean SVL (mm)	Asymptotic size (mm)	Max age (years)	AM (years)	Mean age (years)	k	ESP	SR	References
P. caralitanus	Işıklı Lake, Turkey	M	34	72.62	103.00	8	2	4.53	0.194	5.68	0.81	Present study
		F	38	79.16	115.80	9	2–3	5.03	0.289	6.03	0.82	
	Beyşehir Lake, Turkey	M	35	80.63	117.20	9	3	4.66	0.118	6.62	0.84	
		F	35	89.40	128.80	10	2–3	5.43	0.180	7.19	0.85	
		M	38	75.56	109.00	9	3–4	5.01	0.180			Erişmiş and Chinsamy 2010
		F	51	92.05	126.24	10	3–4	6.01	0.160			
		M	96	90.41	111.35	9	3	5.63		7.52	0.81	Erişmiş 2018
		F	73	98.29	126.50	10	3	6.33		7.94	0.82	
	Karamık Lake, Turkey	M	66	82.33	99.48	7	3	4.86		6.26	0.76	
		F	76	88.36	111.63	8	3	5.30		6.83	0.78	
	Işıklı Lake, Turkey	M	49	73.06	93.68	6	2	3.69		5.50	0.73	
		F	47	82.54	106.72	8	2	4.80		7.01	0.78	
	Eğirdir Lake, Turkey	M	90	86.44	110.12	8	3	5.42		7.32	0.79	
		F	97	94.52	120.28	10	3	6.21		8.20	0.81	
P. bedriagae	Vali Recep Yazıcıoğlu Dam, Denizli, Turkey	M	30	63.01	80.17	9	2	4.59	0.183			Başkale et al. 2018
		F	22	71.20	83.75	9	2	5.20	0.270			
	Süleymanlı Lake, Denizli, Turkey	M	24	68.01	73.80	11	2	6.44	0.371			
		F	25	76.63	82.67	12	2	6.46	0.219			
	Acıgöl Lake, Denizli, Turkey	M	15	63.09	70.04	9	2	5.59	0.370			
		F	17	71.93	80.79	9	2	5.13	0.237			
	Ornaz Valley, Denizli, Turkey	M	14	69.49	78.64	12	2	5.9	0.193			
		F	14	81.14	96.41	12	2	6.8	0.331			
	Pooled localities	M	80	65.78	88.10	12	2	5.65	0.239			
		F	81	74.31	91.50	12	2	5.79	0.346			
	Sülüklü Lake Manisa, Turkey	M	14	56.10	73.20	4	2	2.5	0.300			Çiçek et al. 2011
		F	22	64.50	92.40	5	2	2.95				
		M	51	59.80	120.00	7	2	3.45	0.220			İsmail and Çiçek 2017
		F	76	59.78	137.00	9	2	4.33	0.360			
P. ridibundus	Yıldızlı Stream, Trabzon, Turkey	M	38	64.58		7	3–4	3.90				Yılmaz et al. 2005
		F	11	76.64		6	3–4	3.72				
	Lake Vistonis, Lagos, Greece	M	52	69.03	93.40	5	1	2.96	0.570			Kyriakopoulou-Sklavounou et al. 2008
		F	56	82.38	107.50	5	1	3.73	0.540			
	Milicz Ponds Reserve, Stawno, Poland	M	32	72.20	90.00	6	2	3.7	0.760			Socha and Ogielska 2010
		F	38	79.80	102.30	7	3	4.4	0.590			
	Artvin (Borçka, Lake Karagöl), Turkey	M	20	72.96		8	2	5.15				Gül et al. 2011
		F	25	63.49		7	2	4.20				
	Dörtyol, Hatay, Turkey	M	20	64.70		11	4	5.50				
		F	19	76.74		7	3	5.58				
	Verkhne-Tagil Reservoir, Tagil and Vogulka rivers, Middle Urals	M	–	–	–	–	–	–	–	–	–	Ivanova and Zhigalski. 2011
		F		92.80	116.00	9	2	5.4				
	The Reftinskii Reservoir Reft River, Middle Urals	M	–	–	–	–	–	–	–	–	–	
		F	26	112.90	132.00	8	2	4.4				
	The north of Lorestan Province, central Zagros, Iran	M	26	71.14		11	3	6.43				Ashkavandi et al. 2012
		F	14	74.05		7	3	4.5				
	North-western part of Croatia	M	5			13	3	8				Cavlovic et al. 2018
		F										

Figure 4. Annual growth rate changes of males and females of *P. caralitanus* between populations

Discussion

In this study, we investigated the demographic structure of *P. caralitanus,* using the skeletochronology method which provides information about the individual variation of life history traits and data on growth and age at maturity. According to the results, the age structure of *P. caralitanus* was quite similar in our two different study sites. The results showed similarity with the previous studies (Table 2). In contrast to Erişmiş (2018), we estimated the age of sexual maturity as 2 and 3 years for males and females, respectively. Although Erişmiş (2018) accepted individuals as subadults with an SVL less than

65 mm, we found mature individuals less than 65 mm of SVL in both populations (Table 1). Similar age distributions were observed in other cognate species of *Pelophylax* sp. (*P. ridibundus* and *P. bedriagae*) that occur in Turkey (Table 2). We found the mean and maximum ages of females were higher than those of males which means female individuals live longer than male individuals and this information is consistent with literature (Tsiora and Kyriakopoulou-Sklavounou 2002; Socha and Ogielska 2010; Çiçek et al. 2011; Gül et al. 2011; Erişmiş 2018).

Our results showed that growth rates of *P. caralitanus* were high until sexual maturity and decreased thereafter. The growth coefficients (k) were higher in females than in males as in many amphibian species (Guarino et al. 2003; Patrelle et al. 2012). For both sexes, the growth curve was quite similar to other populations of *P. caralitanus* (Erişmiş and Chinsamy 2010; Erişmiş 2018) and other amphibian species, such as *Rana temporaria* (Ryser 1988), *Rana subaquavocalis* (Platz et al. 1997) and *P. bedriagae* (Çiçek et al. 2011; Başkale et al. 2018). In addition, the growth rate is lower than the values reported for European populations of *P. ridibundus* (Kyriakopoulou-Sklavounou et al. 2008; Socha and Ogielska 2010).

Females were larger than males in both populations like other Anuran species (Shine 1979). We found that individuals of the Burdur population were larger than the Işıklı population in both genders. Similarly, Erişmiş (2018) claimed that the mean body sizes of individuals from the Işıklı population were smaller than the other populations (see Table 2). However, Başkale et al. (2017) also showed body size variations in five populations of *P. caralitanus* (Ağlasun, Suğla, Gölcük, Akburun, Yazıköy populations), these variations being not statistically significant.

In conclusion, we provided data on body size, growth rate, adult life expectancy, survival rate, age at maturity and longevity of *P. caralitanus* from the Burdur and Işıklı Lake populations, using the skeletochronology method. Burdur and Işıklı Lake populations showed differences in body size, ESP and survival rates. These data provide valuable information on life-history traits of this species in order to develop more efficient conservation measures.

Acknowledgements

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